Pharmacology, Biochemistry and Behavior 90 (2008) 517–524

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Enhancing effect of HT008-1 on cognitive function and quality of life in cognitively declined healthy adults: A randomized, double-blind, placebo-controlled, trial

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ARTICLE INFO

Article history:
Received 30 August 2007
Received in revised form 15 March 2008
Accepted 30 March 2008
Available online 11 April 2008

Keywords:
Cognition
Herb
Quality of life
Ginseng
Clinical trial
Memory
HT008-1

ABSTRACT

HT008-1 is one of the most effective multiherb mixtures that have neuroprotective effects in traditional Korean medicine. The purpose of this study was to conduct a clinical trial of the efficacy of HT008-1 on the neuropsychological functioning and quality of life (QoL) in cognitively intact adults. One hundred and eighteen male (n=42) and female (n=76) volunteers who reported no history of dementia or significant neurocognitive impairments and obtained Korean Version of Mini Mental State Examination total scores of at least 24 were examined via an 8-week, randomized, double-blind, fixed-dose, placebo-controlled, parallel group, clinical trial. Participants were randomly assigned to receive either HT008-1 (n=59) or placebo (n=59) for 8 weeks. Efficacy measures consisted of participants' performance scores from pretreatment baseline to those obtained just before termination of treatment on standardized neuropsychological measures from the subsets of Wechsler Memory Scale—III (WMS-III). QoL was assessed by subjective questionnaires WHOQoL-Bref about five categories. Participants who scored in the lower third of the Auditory recognition delayed at baseline and received HT008-1 exhibited improvement on the WMS-III Auditory recognition delayed subtest compared with placebo controls. The HT008-1 group also improved on general health scores in the QoL test.

1. Introduction

Increasing attention is being paid to the quality of life (QoL) as people age. A major factor in determining QoL involves an individual's cognitive capacity. Cognitive function has been regarded as noncritical for maintaining lifespan, but with increasing incidences of neurodegenerative disease, the geriatric population requires special attention. As such, a great deal of research is now focusing on disease prevention, particularly with respect to neurodegenerative diseases. In particular, Alzheimer's disease (AD), Parkinson's disease, dementia with Lewy bodies, and vascular dementia are important clinical conditions with major impacts on aging patients.

In traditional Korean medicine, many herbs classified as *boyak,* meaning 'a tonic' have been used as anti-aging treatments and for improving QoL in the clinics. Many are on the global market as manufactured health foods. Several herbal remedies on the market today claim to improve cognitive functions, such as attention and memory; however, none of these has been approved by the Food and Drug Administration for mild cognitive impairment.

Clinical research that has been conducted on the herbs' effects on cognition and memory function are often inconclusive or ill defined. For example, some researchers have reported that *Ginkgo biloba* and Ginseng can improve cognitive abilities in healthy volunteers (Rai et al., 1991; Kennedy et al., 2000, 2001, 2002) or patients with AD (Kanowski et al., 1996), however, these findings generally have not been supported by well-controlled clinical studies. Moreover *G. biloba* and Ginseng could not provide any beneficial effects on memory performance in healthy adult volunteers (Persson et al., 2004) or in post-menopausal women (Hartley et al., 2004). Studies of healthy subjects who received Ginseng also were inconclusive (Cardinal and Engels 2001). Because individual herbs have not been shown to improve cognitive function in clinical studies, research into multiherb combinations would be desirable to determine if there are synergistic effects. Other than the mixture of Ginseng and *G. biloba*, multiherb combinations have not been tested for their effects on cognitive function in humans.

The purpose of this study was to investigate the effects on cognition and QoL of HT008-1, a multiherb mixture composed of four

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0091-3057/$ – see front matter © 2008 Published by Elsevier Inc.
doi:10.1016/j.pbb.2008.03.033

herbs: the roots of *Panax ginseng*, *Acanthopanax senticosus* (Siberian Ginseng), *Angelica sinensis*, and *Scutellaria baicalensis* (skullcap). HT008-1 was made after screening more than 200 herbs based on *in vivo* data of neuroprotection and memory improvement and on traditional Korean medicine composition theory. Each herb of HT008-1 is known for having anti-aging, adaptogenic and tonic effects, and has been used individually for the treatment of chronic, geriatric, inflammatory, and neurological disorders in traditional Korean medicine (Kim, 2001).

Each herb composing HT008-1 has also been reported to provide neuroprotection and enhance memory. Ginseng (D'Angelo et al., 1986; Zhong et al., 2000; Nishijo et al., 2004), ginsenoside Re, Rg1 (Yamaguchi et al., 1996), and nonsaponin fractions (Kurimoto et al., 2004) enhance cognitive function in rodents. *S. baicalensis* is neuroprotective in ischemic rodents (Kim et al., 2001), and its flavonoids (Shang et al., 2001, 2006), baicalein (Liu et al., 2007) and oroxylin A (Pershina et al., 2005; Kim et al., 2006, 2007), improve memory function in rats. *A. senticosus* provides a neuroprotective effect by inhibiting microglia activation in brain ischemia (Bu et al., 2005) and improves short-term memory in healthy humans (Arushanian et al., 2003). *A. sinensis* and its ferulic acid (50 and 100 mg/kg) enhance memory by activating the cholinergic system in mice (Hsieh et al., 2002).

The standardized form of HT008-1 showed neuroprotective effects in the four-vessel occlusion and middle cerebral artery occlusion models in rats, and the global and focal brain ischemia rat model, and enhanced memory in the scopolamine-induced memory impairment model in mice (Seo et al., 2007; Kim et al., 2008). Each herb of HT008-1 also showed synergistic neuroprotective effects, which means HT008-1 was more effective than any of the individual herbs (our unpublished data). Based on these findings, and the fact that the herbs of HT008-1 have been used as a tonic in traditional Korean medicine, we hypothesized that HT008-1 would improve QoL and cognitive function in humans.

This study was aimed at investigating the effect of HT008-1 on cognitive function including memory enhancement by the neuropsychiatric test batteries composed of 6 primary subtests and 5 optional subtests of the third edition of the Wechsler Memory Scale (WMS-III), designed for assessment of learning and memory in individuals (Wechsler, 1997). In this study, Logical memory I & II, Verbal paired associates I & II of Auditory Memory Subtest, and Letter–Number Sequencing, Digit Span of Attentional Subtest, and Auditory recognition delayed Index were used. We also assessed health related QoL by the brief version of the World Health Organization Quality of Life instrument (WHOQoL-Bref) about five categories (energy and fatigue, sleep and rest, thinking, learning, memory and concentration, negative thought and sexual ability).

2. Methods

2.1. Subjects

Participants were recruited at Kyung Hee University Medical Center through bulletin, Internet, and newspaper advertisements. An initial telephone interview was conducted to determine if the participant was likely to meet entry criteria for the study. Participants were required to have completed six or more years of education and have no difficulty reading or writing. One hundred and fifty male and female volunteers between ages 50 and 70 years, who reported no history of dementia or significant neurocognitive impairment and passed the initial interview were randomized in this study. All participants who chose to participate in this trial completed a written consent form. Additional requirements for inclusion in the study included: a score≥borderline scores of 16.9 at ages 65 to 84 or 18.9 at ages 55 to 64 on the memory subscale of the Korean-Dementia Rating Scale (K-DRS) (Chey et al., 1999) and a score of >24 on the Korean Version of the Mini Mental State Examination (MMSE-K) (Park and Kwon, 1989), a standard tool to assess mental status, including orientation, attention, immediate and short-term recall, language, and the ability to follow simple verbal and written commands(Folstein et al., 1975). Individuals who had histories of neurological disorders, including stroke, head injury, psychiatric disorders (mental retardation, schizophrenia, depression with ≥21 on the Beck's Depression Inventory (BDI) scores), drug abuse, alcohol dependence/abuse, or a disease or surgery that could influence drug absorption, were excluded from this study before the K-DRS test or MMSE-K test.

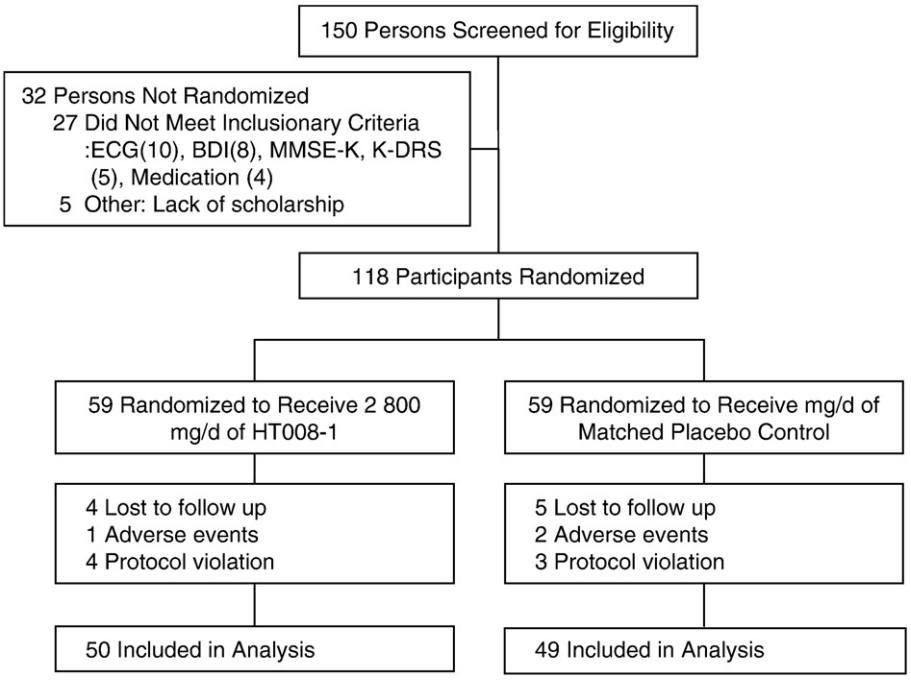

Fig. 1. Trial flow diagram.

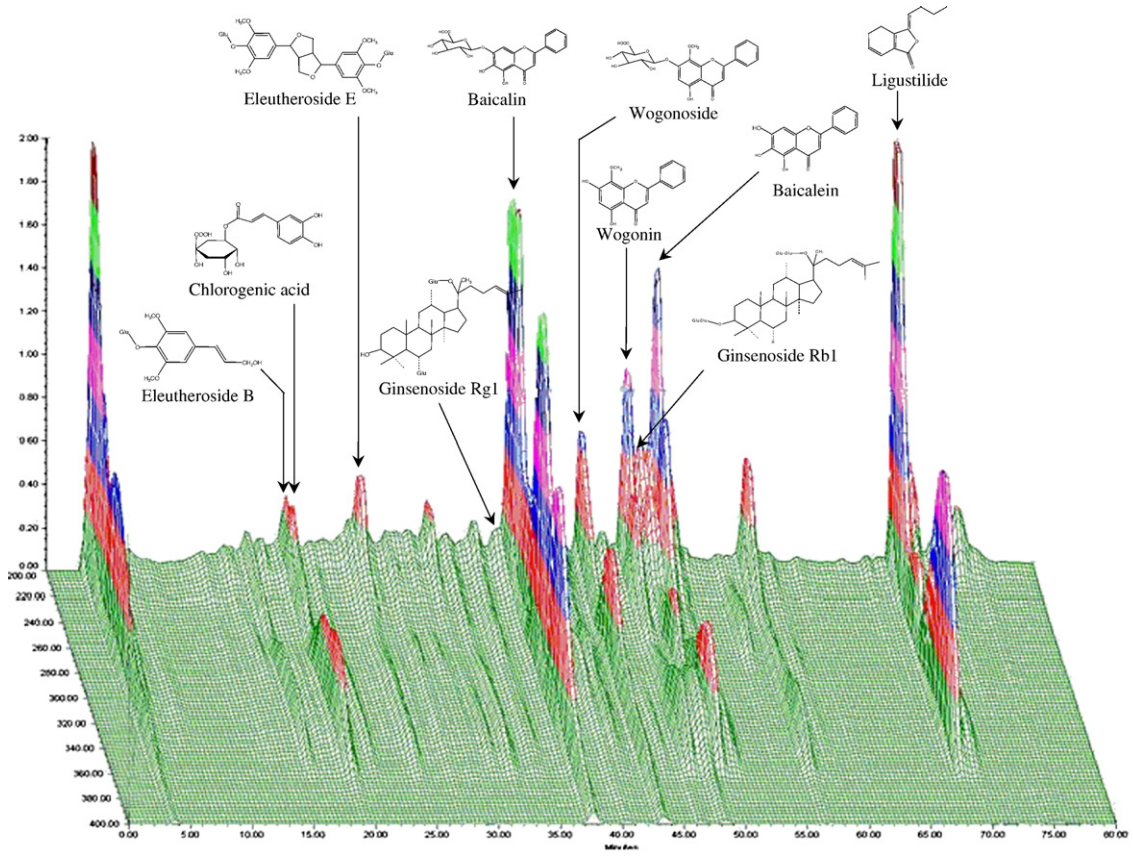

Fig. 2. 3-D HPLC chromatogram of HT008-1. Marker compounds are as follows: ginsenoside Rb1 and Rg1 of *P. ginseng*; baicalin, wogonoside, baicalein and wogonin of *S. baicalensis*; eleuthroside B, E, and chlorogenic acid of *A. senticosus*; ligustilide of *A. sinensis.*

Potential subjects were interviewed, given ECGs, and had blood drawn to screen for total protein, albumin, AST, ALT, GGT, BUN, creatine, and common blood cell counts, including WBC, RBC, hemoglobin, and platelets. Individuals who were being treated with hormones, antidepressants or other psychoactive medications, who had internal medical problems on blood test (except stable hypertension or diabetes mellitus with medication), who had an unstable medical state, were pregnant or would become pregnant, were undernourished, or who drank more than eight cups of coffee per day also were excluded. Finally, participants were excluded if they had participated in other clinical trials in the last month.

Participants meeting the preliminary cognitive and medical inclusion criteria were asked to stop taking health supplements that could influence memory but to continue use of medications for critical diseases (e.g., hypertension, diabetes mellitus). While on the HT008-1 therapy, the participants were asked to keep a medication diary and to bring it with their remaining study medicines every other week in order to assess the compliance and to check for adverse effects.

Of the 150 participants who were screened for eligibility, 32 were excluded due to unstable health state with abnormal ECG or abnormal blood test ($n=10$), >21 on BDI scores ($n=8$), low MMSE-K or memory subscale score of K-DRS ($n=5$), ineligible medication ($n=4$), and shortage of academic background ($n=5$). Of the 118 participants who were initially enrolled in the study, 99 completed the trial's protocol and were available for the efficacy analyses. Among the 19 participants who were excluded, five from the placebo group and four from the HT008-1 group were lost to follow-up. Two from the placebo group and one from the HT008-1 group withdrew prematurely secondary to the adverse events with light dietary problems, such as abdominal pain or diarrhea. Three additional participants from the placebo group and four from the HT008-1 group were excluded due to protocol violations (e.g., noncompliance with the treatment regimen). Fig. 1 shows the trial flow diagram for this study.

There were no significant differences between the HT008-1 and placebo groups in demographic characteristics, current states of medication at baseline, or results of paper questionnaires (i.e., MMSE-K, memory subscale of K-DRS, BDI). Demographic characteristics of the HT008-1 and placebo groups are shown in Table 1.

2.2. HT008-1 preparation

Each herb was identified by Prof. H. Choi at the dept. of Herbal Pharmacology, College of Oriental Medicine, Kyung Hee University,

Table 1
Demographic characteristics in placebo and HT008-1 groups

Characteristics	Placebo ($n=54$)	HT008-1 ($n=55$)
Age	59.0(5.0)	59.4(5.1)†
Sex no. (%)		
Male	19 (35.2)	21 (38.2)
Female	35 (64.8)	34 (61.8)
Education (years)	11.3(2.9)	12.2(3.4)
Memory subscale of K-DRS	22.6(1.4)	22.8(1.7)
MMSE-K	27.1(1.5)	27.3(1.4)
BDI	8.2(5.2)	7.7(4.8)
Current internal use of medicine (no. (%))		
Antihypertensive medication	10 (18.2)	15 (27.8)
Hypoglycemic medication	5 (9.1)	3 (5.6)
Thrombolytic medication	4 (7.3)	4 (7.3)
NSAIDs	13 (23.6)	15 (27.8)
Calcium	4 (7.3)	1 (1.9)
Other medications	8 (14.5)	7 (13.0)

Data are mean (SD).
†Comparison between placebo and HT008-1 by *t*-test.
Other medications were digestive, analgesics, anti-diuretics, nutrients and so on.

Seoul, Korea and The pharmaceutical preparation procedures for HT008-1 (Lot. No.001) and placebo were furnished by the manufacturer (NeuMed Inc., Korea).

Each herb of HT008-1 was mixed as following; *A. senticosus*: *A. sinensis*: *S. baicalensis: P. ginseng*=41.3: 33.0: 3.7: 22.0. At first step, 3 kinds of herb except *P. ginseng* were extracted together with 70% ethanol for 6 h at 82 °C of reflux apparatus. After reflux, the solution was filtered and the filtrate was evaporated by rotary evaporator up to 60°Bx. At second step, *P. ginseng* was separately extracted with at same condition. Extraction procedure for *P. ginseng* was repeated 2 times. After reflux, the filtrate was evaporated up to 60°Bx. At final step, the 2 kinds of concentrates (60°Bx) were mixed for HT008-1. The quantitative authentication of HT008-1 was done by HPLC analysis (Waters 600 pump; column, Hypersil Gold C_{18}, 250 mm×4 mm, 5 μm; eluent, (A) 0.5% H_3PO_4/H2O (v/v) and (B) CH_3CN, a linear gradient from 5% to 50% of (B) in (A) for 60 min and 50% to 70% for 60 to 61 min and holding 70% of (B) in (A) from 61 to 80 min; flow rate, 1 ml/min; detector, PDA; room temp.). HPLC chromatogram of HT008-1 was shown in Fig. 2.

In this study, to standardize and control the herbal extract, six batches of HT008-1 extract were prepared and the content of 4 compounds in each batch was analyzed in triplicate. The content range of the 4 marker compounds in the batches was as follows: eleutheroside E 0.62–0.80 mg/g; baicalin 5.96–8.92 mg/g; ginsenoside Rb1 1.02–1.64 mg/g; ligustilide 2.84–4.36 mg/g. The content of four compounds in 6 batches was quite stable to certify the efficacy. Therefore, the content range was controlled to standardize and to control the quality of HT008-1.

In this clinical study, HT008-1 was prepared as a liquid containing 2600 mg of standardized extracts in a 30-ml pouch of solution. In this case, the HT008-1 liquid was containing the standard compound as follows; eleutheroside E 4.3 mg%; baicalin 74.3 mg%; ginsenoside Rb1 6.8 mg%; ligustilide 26.8 mg%. The placebo, which contained oligosaccharides, caramels, and other materials was also made in 30-ml pouches.

2.3. Study design and procedures

The protocol of this study was approved through peer review by the institutional review board of the Hospital of Oriental Medicine Kyung Hee University Medical Center (KUMC), where the study was conducted. All procedures were performed in accordance with the recommendations of the Korea Good Clinical Practice (KGCP) and World Medical Association Declaration of Helsinki (WMA, 1997) on biomedical research involving human subjects.

This study used an 8-week, randomized, double-blind, placebo-controlled, parallel group study conducted through outpatient visiting. Subjects meeting inclusion criteria were randomly assigned to either HT008-1 (n=59) or placebo (n=59) groups through the online service of www.randomizer.org. HT008-1 was ingested by subject two pouches daily with a daily dose of 5200 mg (an average of 100 mg/kg). All HT008-1 that was used in this project originated from the same batch/lot number. Placebo was also ingested by subject two pouches daily that did not differ in appearance (e.g., color, size, smell, or taste).

Participants were administered a series of neuropsychological and QoL tests immediately before the initiation of HT008-1/placebo therapy, and after 8 weeks of treatment just before the termination of this regimen. All neuropsychological testing sessions were conducted by trained clinical neurophysiologists.

To investigate the effect of HT008-1 on cognitive function, including memory enhancement, subtests of the Wechsler Memory Scale—Third Edition (WMS-III) were used. This test is widely used for assessing learning and memory function in clinical populations (Weschler, 1997). The WMS-III has good reliability in terms of both internal consistency and test–retest reliability in different clinical samples, including AD (Iverson, 2001). It is composed of six primary subtests and five optional subtests. In this study, Logical memory I & II, Verbal paired associates I & II of Auditory memory subtests, and Letter–Number Sequencing, Spatial Span of Attentional subtests were applied. Auditory recognition delayed Index, which represents delayed recall also was applied. Health-related QoL was also assessed using the WHOQoL-Bref (Min et al., 2002). This instrument, which is a measure of health status filtered by perception and expectations of the individual (Testa and Simonson 1996), has gained recognition as a valuable tool for evaluating effects of medical treatments and healthcare services.

The difference in WMS-III or WHOQoL-Bref between baseline and 8 weeks of treatment was evaluated. Blood tests (AST, ALT, BUN/Creatine) were administered at the end of the study to evaluate potential toxicity of HT008-1 to the liver and kidney.

Adverse effects were investigated with classification of gastrointestinal symptoms (e.g., diarrhea, constipation, vomiting etc.), headache and dizziness; sleep disturbance (e.g., insomnia, hypersomnia), dermatitis, pain, and others. The participants who did not visit a study site for these follow-ups were excluded from the study.

Compliance with the treatment regimen was assessed via pack counts conducted on the follow-up day at the end of the 2nd, 4th, 6th, and 8th week of treatment. A deviation of more than 20% from the optimum study treatment regimen was operationally defined as noncompliant. Participants were excluded from the study if they did not take more than 8 packs of study medicines in any 2-week period. When excluded, they were asked to stop taking study medication.

2.4. Outcome measurement

2.4.1. Wechsler Memory Scale—III (WMS-III)

These tasks are standardized subtests of WMS-III and were translated by Korean researchers. Since visual memory could be easily affected by social and cultural background, visual memory subtests were excluded because of the social and cultural differences between Korea and the US.

Administration and scoring of WMS-III was conducted according to published method (Wechsler, 1997). Meetings for inter-rater consistency took place five times before beginning the trial. Administration of the WMS-III subtests required 30–40 min. The subtests of WMS-III used in this trial were as follows: Logical memory I & II, Verbal paired associates I & II of Auditory Memory Subtest, and Letter–Number Sequencing, Digit Span of Attentional Subtest, and Auditory recognition delayed Index, which represents delayed recall.

In Logical memory I, two short stories were orally presented. The second story was presented twice. The examinee was asked to retell the stories from memory. In Logical memory II, the examinee was asked to retell both stories from the immediate condition. Then the examinee was also asked yes/no questions about both stories. In verbal paired associates I, an orally presented task requiring the examinee to learn novel word associations was provided. After 8 word pairs were read, the first word of each pair was then given, and the examinee was asked to provide the corresponding word. There were 4 trials of the same list in different orders. In verbal paired associates II, the examinee was orally presented with the first word of each pair learned in the immediate condition and asked to provide the corresponding word. The examinee was then asked to read a list of 24 word pairs and asked to identify each as either one of the word pairs he or she was asked to remember or a new word pair.

2.4.2. World Health Organization Quality of Life Assessment Instruments-BREF (WHOQoL-Bref)

The multidimensional WHOQoL instrument comprises four items concerning overall QoL and general health as well as 24 facets categorized into four domains: physical, psychological, social relationship, and environmental. Each of the 24 facets has four items, making

Table 2
Efficacy of blinding

Question Options	Placebo (n=49)	HT008-1 (n=50)	p-value[a]
Participant thought he/she was in the placebo group	19 (38.8)	11 (22.0)	0.147
Participant thought he/she was in the HT008-1 group	15 (30.6)	23 (46.0)	
Participant couldn't judge whether he/she was in the HT008-1 group or in the placebo group	15 (30.6)	16 (32.0)	

Data are presented by the number of people (%).
[a] Chi-square analysis.

it a 100-question self-reported questionnaire. The WHOQoL-Bref was derived from 24 facets of WHOQoL. From each facet, the one item that most strongly correlated with the mean of four items was selected. Then two questions about overall QoL and general health were added. WHOQOL-BREF consisted of 2 questions about overall quality of life and general health, 7 about physical heath, 6 about psychological states, 3 about social relationships, and 8 about environmental states.

The test–retest reliability of total score was statistically significant (0.841). The Cronbach α-value of the total score for internal consistency was 0.898. The correlation coefficient in total score between WHOQoL and WHOQoL-Bref was 0.901. This questionnaire uses 5 likert scales. For each domain, a higher score indicates a better QoL. The conversion score of WHOQoL-Bref is distributed from 4 to 20 or from 0 to 100. In this study, a 0-to-100 scoring system was selected.

2.5. Statistics

Since the trial design involved neuropsychological assessments only at pretreatment baseline and just before treatment termination, the protocol data set was utilized in the statistical analyses. To examine changes in performance on the subtests of WMS-III between the HT008-1 and placebo control groups, the differences of baseline scores and post-treatment scores were used as the dependent variables in the efficacy analyses. Since only the pretreatment and post-treatment scores were considered, analysis for efficacy was performed by per-protocol analysis. The differences between pre-treatment and post-treatment scores in each subtest were assessed with either Mann Whitney U Test or Independent Sample t-test. According to the result of Shapiro–Wilk normality test, if the p-value was lower than 0.05, the Mann Whitney U test was used and if not, the Independent Sample t-test was used.

Analyses for the effects on WMS-III and QoL were performed with per-protocol analysis. To limit the normal range of cognitive function in the healthy participants, they were arranged in order of raw Auditory recognition delayed score at baseline. If participants achieved the same score, they were arranged in order of MMSE-K acquired level. Then they were divided into three groups (high 1/3, middle 1/3, low 1/3) according to this score, which represents delayed recall and so was shown as long-term memory ability. The comparison between the HT008-1 and placebo groups was also conducted in the lower third and upper third groups.

The demographic variables, age (years), education (years), memory subscale of K-DRS, MMSE-K, and BDI were analyzed by Mann Whitney U Test if the p-value from Shapiro–Wilk normality test was lower than 0.05; otherwise the Independent Sample t-test was used. To examine any differences that may have existed between the participants in the HT008-1 and placebo groups who were available for the efficacy analyses, Chi-square tests were conducted on the following descriptive and criterion measures: the ratio of sex, age in years, the educational level in years, MMSE-K total scores and BDI. Data from the 118 participants who were initially randomized in this study were used in the safety analyses. Fisher's Exact Test was used to analyze the ratio of adverse events.

To analyze the blinding efficacy, participants were asked to which group they belonged. Chi-square analysis showed that there were no significant differences of the self suggestion between HT008-1 and placebo groups. From this result, we concluded that blinding was not broken (Table 2).

SPSS 13.0 for windows (SPSS Inc., Chicago, Il, USA) was used for all analyses. Results were considered statistically significant if the p values were <0.05.

3. Results

3.1. WMS-III

Table 3 provides a summary of the groups' performance score at baseline and week 8 within groups for each neuropsychological test variable of WMS-III subtests and WHOQoL-Bref. In WMS-III subtests, the HT008-1 group demonstrated significant improvement in Logical memory I (p=0.006), Logical memory II (p=0.008), Verbal paired associates I (p=0.001), and Auditory recognition delayed (p=0.075), but there was no significant difference in Verbal paired associates II, Letter–Number Sequencing, or Spatial Span in this group. In the

Table 3
WMS-III subtests and WHOQoL-Bref at baseline and week 8 within groups

Test	Range of scores	Placebo (n=49)		p-value	HT008-1 (n=50)		p-value[a]
	Baseline	Week 8	Baseline		Week 8		
WMS-III							
Logical memory I	1–19	6.98(3.1)	8.78(3.2)	0.006[a**]	6.90(3.1)	8.90(3.6)	0.006[b**]
Logical memory II	1–19	8.16(3.5)	10.33(3.2)	0.002[b**]	8.38(3.6)	10.46(3.5)	0.008[b**]
Verbal paired associates I	1–19	8.29(2.7)	10.29(3.2)	0.001[a**]	8.56(2.9)	10.10(3.2)	0.001[a**]
Verbal paired associates II	1–19	8.22(2.5)	9.84(2.7)	0.003[b**]	8.92(3.2)	9.94(2.8)	0.075[b]
Letter–Number Sequencing	1–19	8.86(3.3)	10.00(3.0)	0.079[a]	8.42(2.7)	8.80(3.0)	0.485[b]
Spatial span	1–19	10.08(3.6)	10.45(4.0)	0.633[a]	9.58(3.7)	9.72(3.4)	0.931[b]
Auditory recognition delayed	1–19	8.06(2.8)	9.86(3.3)	0.005[a**]	7.94(3.5)	10.12(3.2)	0.005[a**]
WHOQoL-Bref							
Overall quality of life	1–5	3.67(0.8)	3.67(0.6)	0.819[b]	3.60(0.6)	3.78(0.6)	0.101[b]
General health	1–5	3.61(0.8)	3.39(0.8)	0.236[b]	3.44(0.7)	3.56(0.6)	0.293[b]
Domain 1 (physical health)	0–100	63.63(15.7)	62.29(13.4)	0.560[b]	61.88(14.3)	64.32(12.5)	0.321[b]
Domain 2 (psychological health)	0–100	52.45(14.8)	55.69(15.8)	0.397[b]	52.96(13.6)	58.58(11.8)	0.297[a]
Domain 3 (social relationships)	0–100	53.94(16.3)	56.86(14.3)	0.309[b]	52.22(19.7)	59.16(11.5)	0.134[b]
Domain 4 (environment)	0–100	54.69(13.9)	54.49(15.7)	1.000[b]	55.22(13.0)	55.62(10.9)	0.953[b]

Data are means (SD).
*: $p<0.05$ **: $p<0.01$.
[a] Independent sample t-test.
[b] Mann Whitney U test.

Table 4
Comparison of subtests of WMS-III and QoL between the HT008-1 and placebo group at the end of the study in the total, higher third, and lower third groups divided by the baseline score of auditory recognition delayed, which represents long-term memory

Test	Total	The higher group	The lower group†
WMS-III			
Logical memory I	0.738†	0.163†	0.393†
Logical memory II	0.893†	0.541†	0.333†
Verbal paired associates I	0.358†	0.812†	0.402†
Verbal paired associates II	0.147†	0.250†	0.313†
Letter–Number Sequencing	0.144†	0.156†	0.844†
Spatial span	0.653†	0.382†	0.952†
Auditory recognition delayed	0.504†	0.354†	0.042†*
WHOQoL-Bref			
Overall quality of life	0.184†	0.376‡	0.055†
General health	0.043†*	0.620‡	0.043‡*
Domain 1 (physical health)	0.143†	0.903†	0.022†*
Domain 2 (psychological health)	0.285†	0.703†	0.003†**
Domain 3 (social relationships)	0.277†	0.362‡	0.035‡*
Domain 4 (environment)	0.761†	0.643†	0.171†

Data are p values between placebo(week 8 minus baseline) and HT008-1(week 8 minus baseline).
†: Independent sample *t*-test.
‡: Mann Whitney *U* test.
*: $p<0.05$ **: $p<0.01$.

placebo group, the Logical memory I ($p=0.006$), Logical memory II ($p=0.002$), Verbal paired associates I ($p=0.001$), Verbal paired associates II ($p=0.003$), and Auditory recognition delayed showed significant improvement ($p<0.005$), but other WMS-III subsets did not show any major improvement.

To examine changes in performance on the neuropsychological measures over time between the HT008-1 group and placebo control groups, participants' raw change in performance scores from the pretreatment baseline to week 8 were used as the dependent variables in the efficacy analyses. Analysis of WMS-III subtests for the cognitive function of the 99 participants who completed the trial indicated that there were no significant differences between HT008-1 and placebo group. However, in the lower third group, there were significant differences of Auditory recognition delayed subtest ($p=0.042$) (Table 4). The HT008-1 group exhibited improvement on memory in the lower third rather than in the upper third of participants who were divided by baseline score of Auditory recognition delayed.

3.2. Quality of life

No significant improvement at the groups' performance score between baseline and week 8 within groups was seen in either the HT008-1 group or placebo group (Table 3). In the analysis of WHOQoL-Bref, participants' raw change in scores on the WHOQoL-Bref over time from the pretreatment baseline to week 8 was also used as the dependent variables in the efficacy analyses. The participants who received HT008-1 for 8 weeks exhibited significant improvement in General Health compared with the placebo group (Table 4). Other subtests of the WHOQoL-Bref in the total or the higher did not show significant differences between the HT008-1 and the placebo group (Table 4). However, in the lower third group, there were significant differences in General health ($p=0.043$), Domain I (physical health; $p=0.022$), II (psychological health; $p=0.003$), and III (social relationship; $p=0.035$) of WHOQoL-Bref (Table 4).

3.3. Safety

Twelve adverse events were reported by the placebo group and 14 adverse events were reported by the HT008-1 group. However, no serious adverse events were reported during the study. All of the adverse events that were reported were rated as either mild or mild to moderate in intensity, and no causal relationship was determined between the HT008-1 treatment and any adverse event. Of the six adverse events related to the nervous system, five were headache and dizziness and one was episodes of pain. Gastrointestinal adverse events consisted of episodes of gastrointestinal upset, including diarrhea, constipation, vomiting, and gastric complaints. Dermatologic system events were dermatitis or eczema. Two adverse events that occurred in the placebo group, eyeball fatigue and tiredness, were reported. Overall, there was no significant difference between the HT008-1 and placebo group (Table 5).

4. Discussion

The present study was a carefully controlled double-blind, placebo-controlled, randomized clinical trial to examine the efficacy of a multiherb mixture on the neuropsychological function and QoL of cognitively intact adults. The primary findings of this study indicated that 99 participants who received 5200 mg of HT008-1 daily for 8 weeks showed no significant differences with the placebo group in WMS-III subtests, however, participants in the lower third group of the Auditory recognition delayed score at baseline exhibited significant improvement on the WMS-III Auditory recognition delayed subtest by treatment end compared with the placebo controls. The QoL of participants in the HT008-1 group also significantly improved in terms of general health scores. This study is the second clinical trial of HT008-1. In the previous trial, the cognitive function of HT008-1 was evaluated by Korean-California Verbal Learning Test (K-CVLT), Korean-Complex Figure Test (K-CFT), Corsi-block test, and Rey–Kim Complex Figure Test, but the results did not show significant improvement in the HT008-1 group (unpublished data). There were two limitations in the previous trial, practice effect and ceiling effect. The repetitive examinations caused both the experimental group and placebo control group to show improvement on K-CVLT scores during the 8 weeks. K-CVLT was also considered to have limitations for clinical trial of healthy subjects because many of the participants made perfect K-CVLT scores after repeating two or three times.

Memory testing is an important component in a comprehensive neuropsychological evaluation. In the current trial, WMS-III, the most commonly used battery for memory testing(McDowell et al., 2004), was selected for investigating recognition. WMS-III, widely used for emphasis on the construct of delayed memory that had been done in previous editions (Tulsky and Ledbetter, 2000), has good reliability in terms of both internal consistency and test–retest reliability in different clinical samples including Alzhermer's disease (Iverson, 2001). The participants were tested two times, at baseline and after 8 weeks, to overcome the first trial's limitations of practice and ceiling effects. In the WMS-III subtests, the HT008-1 group demonstrated improvement in Logical memory I, Logical memory II, Verbal paired associates I, and Auditory recognition delayed after 8 weeks of treatment, but the placebo group also showed significant improvements in the same subsets. The results do not demonstrate a difference in cognitive function between the HT008-1 and placebo groups.

It is very difficult to limit the normal range of cognitive function because the cognitive function of healthy people is much more widely distributed than that of diseased people (Zappala et al., 1995) Most neuropsychological tests are also focused on discrimination of abnormality or severity of disorders rather than the definition of normalcy. For these reasons, despite some clinical trials that have reported on the cognitive function of herbal extracts in healthy subjects, none of these studies have provided convincing evidence of cognitive improvement (Le Bars et al., 1997; Kreijkamp-Kaspers et al., 2004).

To decrease the range of cognitive function of healthy subjects, the participants were arranged in order of raw WMS-III Auditory recognition delayed score at baseline, which represents delayed recall

and so indicates long-term memory ability. Then the participants were divided into three groups according to this score, the upper, the middle, and the lower. Interestingly, the lower third group of Auditory recognition delayed score at baseline receiving HT008-1 demonstrated significant cognitive improvement compared with the placebo group in the WMS-III Auditory recognition delayed subtest after 8 weeks treatment, but the other two groups did not.

We believe that cognitive improvement was seen only in the lower third group for two reasons. (1) The lower third group belonged to a borderline group between normal and abnormal cognitive function. The individuals in this group could be in cognitive decline or have mild cognitive impairment, even though they belonged to a normal group. This would suggest that HT008-1 improves cognitive function in people in cognitive decline. (2) The cognitive improvement in the lower third group was easily evaluated for pharmacological effect because they were below the normal range.

The cognitive improvement afforded by HT008-1 is supported by previous studies showing cognitive improvement by the individual herbs contained in HT008-1 in animals or humans. Ginseng, a major herbs of HT008-1, enhance cognitive performance by hippocampal formation (Zhong et al., 2000; Nishijo et al., 2004), psychomotor performance (D'Angelo et al., 1986), glucoregulatory properties (Reay et al., 2006) in rodents, or in aged rats (Nitta et al., 1995) (Kurimoto et al., 2004). Ginsenoside Re and Rg1 are also effective on impaired performance caused by scopolamine (Yamaguchi et al., 1996). The roots of *S. baicalensis* (Kim et al., 2001) and its compounds, oroxylin A (Kim et al., 2006) and baicalein (Liu et al., 2007) has neuroprotective effect by antioxidation, inhibiting microglia activation and increasing BDNF expression in ischemic rodents. Total flavonoids of *S. baicalensis* and oroxylin A respectively improve memory deficits induced by 2-VO rats (Shang et al., 2006), chronic galactose (Shang et al., 2001), or scopolamine. The root and stem bark of *A. senticosus* has a neuroprotective effect by inhibiting inflammation and microglial activation in ischemic rats(Bu et al., 2005), and improves short-term memory in healthy humans (Arushanian et al., 2003). The root of *A. sinensis* and its compounds, ferulic acid reverses the step-through latency shortened by scopolamine and cycloheximide (Hsieh et al., 2000).

Regarding the clinical study, the beneficial effects of these herbs on memory are less well defined. In a randomized double-blind placebo-controlled study, post-menopausal women aged 51–66, randomly assigned to 12 weeks' chronic administration of Gincosan (320 mg/day), containing 120 mg *G. biloba*, and 200 mg *P. ginseng* (n=30), appeared to have no beneficial effects in post-menopausal women (Hartley et al., 2004). Twenty healthy subjects participated in a placebo-controlled, double-blind study receiving ginseng, only at the dosage of 400 mg ginseng showed partly effects and inconclusive. Because any component herb of HT008-1 have not been clearly reported to enhance memory and cognitive function in human, the cognitive improvement of HT008-1 shown in the study would result from synergistic interaction of each of the HT008-1. Future research is required to extend the present study's findings on the mechanism of HT008-1 and the synergistic effects of the individual herbs.

There are a number of limitations to this study. First, the normal range of cognitive variation is very large. Limiting the normal range will be needed in clinical trials of healthy subjects because the participants are usually categorized by the severity of illness in clinical studies of patients. The second limitation of the study is the practice effect, as described for the previous study, because the same tests were given to the participants. They might remember the task procedures and the actual stimulus materials even after 8 weeks.

Another shortcoming of this study involved the neuropsychological tests. First, WMS-III was not standardized in Korea, so American data was used for modifying the raw scores. Second, the visual memory test could not be applied because of cultural differences. Third, there was a problem related to standardization. For future clinical trials, development of a memory test standardized in Korea and also widely used all over the world is needed. It is also very important to select the appropriate neuropsychological tests that can assess patients' function exactly. In similar future clinical trials concerning memory enhancement of HT008-1, limits will need to be set for real subjects of borderline or less-than-average memory scores among the healthy volunteers.

A wide variety of instruments exist to measure QoL, all of which have strengths and weaknesses. In this study, QoL was measured using the WHOQoL-Bref, a validated 24-item instrument developed from the original 100-item WHOQoL. This instrument has the advantages of recent international development. HT008-1 significantly improved the participants' quality of life in the general health scores. This result supports that each herb of HT008-1 has been used as tonic in traditional Korean medicine.

Taken together, the results from WMS-III subsets for cognitive function and WHOQoL-Bref indicate that HT008-1 would be beneficial for cognitive improvement in healthy people, especially those in cognitive decline. HT008-1 also improves the quality of life in general health.

Acknowledgements

This work was supported by a grant (PF 0320201-00) of Plant Diversity Research Center of 21st Century Frontier Research Program (Ministry of Science and Technology, Korea), and by grants from the Seoul R&D Program (10524) and the Second Stage of Brain Korea 21 Project (Ministry of Education, Korea).

References

Arushanian EB, Baida OA, Mastiagin SS, Popova AP, Shikina IB. Effect of eleutherococcus on short-term memory and visual perception in healthy humans. Eksp Klin Farmakol 2003;66:10–3.

Bu Y, Jin ZH, Park SY, Baek S, Rho S, Ha N, et al. Siberian ginseng reduces infarct volume in transient focal cerebral ischaemia in Sprague–Dawley rats. Phytother Res 2005;19:167–9.

Cardinal BJ, Engels HJ. Ginseng does not enhance psychological well-being in healthy, young adults: results of a double-blind, placebo-controlled, randomized clinical trial. J Am Diet Assoc 2001;101:655–60.

Chey J, Na DR, Park S, Park E, Lee S. Effects of education in dementia assessment: evidence from standardizing the Korean-Dementia Rating Scale. Clin Neuropsychol 1999;13:293–302.

D'Angelo L, Grimaldi R, Caravaggi M, Marcoli M, Perucca E, Lecchini S, et al. A double-blind, placebo-controlled clinical study on the effect of a standardized ginseng extract on psychomotor performance in healthy volunteers. J Ethnopharmacol 1986;16:15–22.

Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.

Hartley DE, Elsabagh S, File SE. Gincosan (a combination of *Ginkgo biloba* and *Panax ginseng*): the effects on mood and cognition of 6 and 12 weeks' treatment in post-menopausal women. Nutr Neurosci 2004;7:325–33.

Hsieh MT, Lin YT, Lin YH, Wu CR. Radix *Angelica sinensis* extracts ameliorate scopolamine- and cycloheximide-induced amnesia, but not *p*-chloroamphetamine-induced amnesia in rats. Am J Chin Med 2000;28:263–72.

Hsieh MT, Tsai FH, Lin YC, Wang WH, Wu CR. Effects of ferulic acid on the impairment of inhibitory avoidance performance in rats. Planta Med. 2002;68:754–6.

Iverson GL. Interpreting change on the WAIS-III/WMS-III in clinical samples. Arch Clin Neuropsychol 2001;16:183–91.

Kanowski S, Herrmann WM, Stephan K, Wierich W, Horr R. Proof of efficacy of the *Ginkgo biloba* special extract EGb 761 in outpatients suffering from mild to moderate primary degenerative dementia of the Alzheimer type or multi-infarct dementia. Pharmacopsychiatry 1996;29:47–56.

Kennedy DO, Scholey AB, Wesnes KA. The dose-dependent cognitive effects of acute administration of *Ginkgo biloba* to healthy young volunteers. Psychopharmacology (Berl) 2000;151:416–23.

Kennedy DO, Scholey AB, Wesnes KA. Differential, dose dependent changes in cognitive performance following acute administration of a *Ginkgo biloba*/*Panax ginseng* combination to healthy young volunteers. Nutr Neurosci 2001;4:399–412.

Kennedy DO, Scholey AB, Wesnes KA. Modulation of cognition and mood following administration of single doses of *Ginkgo biloba*, ginseng, and a ginkgo/ginseng combination to healthy young adults. Physiol Behav 2002;75:739–51.

Kim H. Textbook of herbal pharmacology. Seoul: Jipmoondang; 2001.

Kim YO, Leem K, Park J, Lee P, Ahn DK, Lee BC, et al. Cytoprotective effect of *Scutellaria baicalensis* in CA1 hippocampal neurons of rats after global cerebral ischemia. J Ethnopharmacol 2001;77:183–8.

Kim DH, Jeon SJ, Son KH, Jung JW, Lee S, Yoon BH, et al. Effect of the flavonoid, oroxylin A, on transient cerebral hypoperfusion-induced memory impairment in mice. Pharmacol Biochem Behav. 2006;85:658–68.
Kim DH, Jeon SJ, Son KH, Jung JW, Lee S, Yoon BH, et al. The ameliorating effect of oroxylin A on scopolamine-induced memory impairment in mice. Neurobiol Learn Mem 2007;87:536–46.
Kim YT, Yi YJ, Kim MY, Bu Y, Jin ZH, Choi H, et al. Neuroprotection and enhancement of spatial memory by herbal mixture HT008-1 in a rat global brain ischemia model. Am J Chin Med 2008;36:287–99.
Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A, Lampe JW, et al. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. JAMA 2004;292:65–74.
Kurimoto H, Nishijo H, Uwano T, Yamaguchi H, Zhong YM, Kawanishi K, et al. Effects of nonsaponin fraction of red ginseng on learning deficits in aged rats. Physiol Behav 2004;82:345–55.
Le Bars PL, Katz MM, Berman N, Itil TM, Freedman AM, Schatzberg AF. A placebo-controlled, double-blind, randomized trial of an extract of *Ginkgo biloba* for dementia. North American EGb Study Group. JAMA 1997;278:1327–32.
Liu C, Wu J, Gu J, Xiong Z, Wang F, Wang J, et al. Baicalein improves cognitive deficits induced by chronic cerebral hypoperfusion in rats. Pharmacol Biochem Behav 2007;86:423–30.
McDowell BD, Bayless JD, Moser DJ, Meyers JE, Paulsen JS. Concordance between the CVLT and the WMS-III word lists test. Arch Clin Neuropsychol 2004;19:319–24.
Min SK, Kim KI, Lee CI, Jung YC, Suh SY, Kim DK. Development of the Korean versions of WHO quality of life scale and WHOQOL-BREF. Qual Life Res 2002;11:593–600.
Nishijo H, Uwano T, Zhong YM, Ono T. Proof of the mysterious efficacy of ginseng: basic and clinical trials: effects of red ginseng on learning and memory deficits in an animal model of amnesia. J Pharmacol Sci 2004;95:145–52.
Nitta H, Matsumoto K, Shimizu M, Ni XH, Watanabe H. *Panax ginseng* extract improves the performance of aged Fischer 344 rats in radial maze task but not in operant brightness discrimination task. Biol Pharm Bull 1995;18:1286–8.
Park JH, Kwon YC. Korean Version of Mini Mental State Examination (MMSE-K) Part I: development of the test for the elderly. J Korean Neuropsychiatr Assoc 1989;28:125–35.
Pershina OV, Skurikhin EG, Suslov NI. The influence of natural preparations on long-term conflict situation consequences in rats with different cognitive abilities. Eksp Klin Farmakol 2005;68:16–8.
Persson J, Bringlov E, Nilsson LG, Nyberg L. The memory-enhancing effects of Ginseng and *Ginkgo biloba* in healthy volunteers. Psychopharmacology (Berl) 2004;172:430–4.
Rai GS, Shovlin C, Wesnes KA. A double-blind, placebo controlled study of *Ginkgo biloba* extract ('tanakan') in elderly outpatients with mild to moderate memory impairment. Curr Med Res Opin 1991;12:350–5.
Reay JL, Kennedy DO, Scholey AB. Effects of *Panax ginseng*, consumed with and without glucose, on blood glucose levels and cognitive performance during sustained 'mentally demanding' tasks. J Psychopharmacol 2006;20:771–81.
Seo J, Woo SY, Kim YT, Kim MY, Park YM, Jin ZH, et al. Enhancing effect of multiherb extracts HT008-1 on memory and cognitive function. Korean J Herbology 2007;22:51–8.
Shang YZ, Gong MY, Zhou XX, Li ST, Wang BY. Improving effects of SSF on memory deficits and pathological changes of neural and immunological systems in senescent mice. Acta Pharmacol Sin 2001;22:1078–83.
Shang YZ, Miao H, Cheng JJ, Qi JM. Effects of amelioration of total flavonoids from stems and leaves of *Scutellaria baicalensis* Georgi on cognitive deficits, neuronal damage and free radicals disorder induced by cerebral ischemia in rats. Biol Pharm Bull 2006;29:805–10.
Testa MA, Simonson DC. Assessment of quality-of-life outcomes. N Engl J Med 1996;334:835–40.
Tulsky DS, Ledbetter MF. Updating to the WAIS-III and WMS-III: Conditions for research and clinical practice. Psychol Assess 2000;12:253–62.
Wechsler D. WMS-III administration and scoring manual. San Antonio, TX: The Psychological Coporation; 1997.
World Medical Association. Recommendations guiding physicians in biomedical research involving human subjects. JAMA 1997;277:925–6.
Yamaguchi Y, Higashi M, Kobayashi H. Effects of ginsenosides on impaired performance caused by scopolamine in rats. Eur J Pharmacol 1996;312:149–51.
Zappala G, Measso G, Cavarzeran F, Grigoletto F, Lebowitz B, Pirozzolo F, et al. Aging and memory: corrections for age, sex and education for three widely used memory tests. Ital J Neurol Sci 1995;16:177–84.
Zhong YM, Nishijo H, Uwano T, Tamura R, Kawanishi K, Ono T. Red ginseng ameliorated place navigation deficits in young rats with hippocampal lesions and aged rats. Physiol Behav 2000;69:511–25.